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14. ABSTRACT "Dielectric Properties and Electrical Breakdown of Biological Membranes" was the topic of a workshop at the Conference on Electrical Insulation and Dielectric Phenomena on October 20, 2002, in Mexico. The workshop, supported by the IEEE Dielectrics and Electrical Insulation Society, the US AFOSR, and ONR, was organized by Karl H. Schoenbach. The presentations focused on nonthermal biological effects caused by high power pulsed electric fields. The interest in this topic was stimulated by recent observations of modifications in cell functions and induction of apoptosis in cells when exposed to ultrashort electrical pulses. Prominent scientists from various countries presented invited talks on membrane structures and functions, dielectric properties, electroporation, and applications. The funding was utilized to support the travel expenses of invited speakers. A special issue on "Dielectric Properties and Electrical Breakdown of Biological Membranes" with J. Weaver (MIT) and K.H. Schoenbach (ODU) will be published (in October 2003) in the IEEE Transactions on Dielectrics and Electrical Insulation.				
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**Final Report
on the Project**

**“Workshop on Dielectric Properties and
Electrical Breakdown of Biological
Membranes”**

Grant No: AFOSR F49620-02-1-0110

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submitted to:

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Abstract

“Dielectric Properties and Electrical Breakdown of Biological Membranes” was the topic of a one-day workshop at the Conference on Electrical Insulation and Dielectric Phenomena on October 20, 2002, in Cancun, Mexico. The workshop, which was supported by the IEEE Dielectrics and Electrical Insulation Society, the US Air Force Office of Scientific Research, and the Office of Naval Research, was organized by Karl H. Schoenbach, director of the Center for Bioelectrics at Old Dominion University. The presentations and discussions focused on nonthermal biological effects caused by high power pulsed electric fields. The interest in this topic was stimulated by recent observations of modifications in cell functions and induction of apoptosis in cells when exposed to ultrashort electrical pulses. Prominent scientists from the United States, Japan, Germany and Israel presented invited talks on membrane structures, membrane functions, dielectric properties, and electroporation. In addition, an overview of applications of both reversible and irreversible electroporation in medicine and biology was presented. This included gene therapy, cancer treatment, biofouling prevention, and bacterial decontamination. The workshop concluded with a panel discussion. The funding provided by AFOSR was utilized to support the travel expenses of invited speakers. As a follow-up to the workshop, a special issue on “Dielectric Properties and Electrical Breakdown of Biological Membranes” with J. Weaver (MIT) and K.H. Schoenbach (ODU) will be published in October 2003 in the IEEE Transactions on Dielectrics and Electrical Insulation.

Introduction

The membrane surrounding biological cells consists of a lipid bilayer with a thickness of approximately 5 nm. The dielectric constant is about 5, and its membrane resistivity is $2 \times 10^9 \Omega \text{cm}$ [1]. The function of the cell membrane is to isolate regions of different materials, but also to facilitate the flow of selected types of ions and molecules from one region to the other. This flow of ions passes through membrane channels, consisting of proteins, which are embedded in the membrane. These ion channels are either chemically (ligand) or electrically (voltage) controlled.

In the resting state, a constant voltage of approximately 70 mV (for mammalian cells) is applied across the membrane. The potential gradient is caused by differences in sodium and potassium ion concentrations inside and outside the cell. For excitable cells, such as nerve and muscle cells, relatively small depolarizing changes in the membrane voltage, on the order of 10 to 20 mV, lead to strong nonlinear changes in the membrane conductance, an effect which allows electrical signal propagation along the axons of neurons. It was studied and modeled by Hodgkin and Huxley in the 1950s [2], who received the Nobel prize for their research in 1962.

A transmembrane voltage of 70 mV corresponds to an electric field of 140 kV/cm. In experiments with artificial membranes free of proteins (channels), 180 mV could be applied before changes in the conductance were observed [3]. This voltage corresponds to a dielectric strength of 360 kV/cm. Higher dielectric strengths can be achieved when pulsed voltages are applied. Generally, for electroporation to occur, which is a kind of electrical breakdown of the membrane, voltages of 1 V need to be applied across the membrane [4] corresponding to dielectric strengths of 2 MV/cm. This holds true for a pulse duration in the microsecond range. For pulses with submicrosecond duration, this value is even higher.

An understanding of the dielectric properties of plasma membranes, and the changes in conductance with increasing voltage, are not only important for biologists, but might offer opportunities for physicists and engineers interested in novel dielectric materials. The facts that artificial membranes can easily be generated, and features such as self-healing effects (reversible electroporation), make them an interesting object of study for the dielectrics and electrical insulation community.

{In addition to effects on the outer membrane, new research results indicate that it is possible to affect subcellular structures [5]. Although this topic was only being presented in one of the ten invited talks, it generated so much interest in the audience that almost all of the discussion centered on this new topic of "Intracellular Electromanipulation".}

References

- [1]. I. Ermolina, Y. Polevaya, Y. Feldman, B.Z. Ginzburg, and M. Schlesinger, "Study of Normal and Malignant White Blood Cells by Time Domain Dielectric Spectroscopy," *IEEE Trans. Dielectrics and Electrical Insulation* **8**, 253 (2001).
- [2]. A.L. Hodgkin and A.F. Huxley, "A Quantitative Description of Membrane Current and its Application to Conduction and Excitation in Nerve," *J. Physiol.* **117**, 500 (1952).
- [3]. T.J. Lewis, "Electronic Processes in Biology," *Phys. Med. Biol.* **27**, 335 (1982).
- [4]. J.C. Weaver, "Electroporation of Cells and Tissues," *IEEE Trans. Plasma Science* **28**, 24 (2000).
- [5]. Karl H. Schoenbach, Stephen J. Beebe, and E. Stephen Buescher, "Intracellular Effect of Ultrashort Pulses," *J. Bioelectromagnetics* **22**, 440 (2001).

Technical Program

The workshop encompassed invited talks, contributed papers (poster-papers), and a discussion session. The program included invited talks on the structure of biological membranes, electrical breakdown (electroporation), measurements of dielectric properties of membranes, modeling of electroporation, subcellular effects caused by nanosecond electrical pulses, and medical and environmental applications of electroporation and intracellular electromanipulation. The presentations were followed by a discussion session, where the invited speakers and representatives of funding agencies served as panelists. Much of the discussion had focused on the effect of ultrashort electrical pulses on biological cells, where the most recent research results were presented by E. Stephen Buescher. A poster session, as part of the general DEIS program on Monday, October 21 with contributed papers, complemented the invited talks (attachment).

Speakers and Topics

1. **Hiidenori Akiyama and Sunao Katsuki**, Kumamoto University, Japan, "Environmental Applications of Biological Inactivation by Pulsed Electric Field and/or Pulsed Discharges in Water"
2. **E. Stephen Buescher**, Eastern Virginia Medical School, USA, "Intracellular Electromanipulation of Mammalian Cells with Submicrosecond Pulsed Electric Fields"
3. **Yuri Feldman**, Hebrew University, Jerusalem, Israel, "Dielectric Properties of Biological Systems; from Amino Acids to Cells"
4. **Ravindra. P. Joshi**, Old Dominion University, USA, "Dynamical Modeling of Cellular Response to Short High-Intensity Electric Fields"
5. **Raphael C. Lee**, University of Chicago, USA "Trauma and Injury Resulting From Strong Electric Field Exposure"
6. **Dietmar P. Rabussay**, Genetronics, Inc, San Diego, USA, "Medical Applications of Electropemeabilization of Cell Membranes"
7. **Christoph Schultheiss**, Forschungszentrum Karlsruhe GmbH, Germany, "Industrial Applications of Electroporation"
8. **HT Tien and A. Ottova**, Michigan State University, USA, "Electrical and Dielectric Properties of Experimental Lipid Bilayers in Relation to Biotechnology"
9. **James C. Weaver**, Harvard-MIT Division of Health Sciences and Technology, USA, "Electroporation of Biological Membranes from Multicellular to Nano Scales"
10. **Ahmed E. Yousef, C. Patrick Dunne, Ragip Unal and Beatrice H. Lado**, Ohio State University, USA, "Damage of Bacterial Cell Membranes by Pulsed Electric Field Treatment."

Workshop on “Dielectric Properties and Electrical Breakdown of Biological Membranes”

7.30 am	Registration
8.15 am	Introduction (Karl H. Schoenbach)
8.20 am	James C. Weaver , Harvard-MIT Division of Health Sciences and Technology, USA, “Electroporation of Biological Membranes from Multicellular to Nano Scales”
9.00 am	HT Tien , Michigan State University, USA, “Electrical and Dielectric Properties of Experimental Lipid Bilayers in Relation to Biotechnology”
9.30 am	Yuri Feldman , Hebrew University, Israel, “Dielectric Properties of Biological Systems; from Amino Acids to Cells”
10.00 am	Break
10.30 am	Raphael C. Lee , University of Chicago, USA, “Trauma and Injury Resulting from Strong Electric Field Exposure”
11.00 am	Ravindra. P. Joshi , Old Dominion University, USA, “Dynamical Modeling of Cellular Response to Short High-Intensity Electric Fields”
11.30 am	E. Stephen Buescher , Eastern Virginia Medical School, USA, “Intracellular Electromanipulation of Mammalian Cells with Submicrosecond Pulsed Electric Fields”
12.00 am	Lunch-Break
1.30 pm	Dietmar P. Rabussey , Genetronics, Inc, San Diego, USA, “Medical Applications of Electroporation of Cell Membranes”
2.00 pm	Ahmed E. Yousef , Ohio State University, USA, “Damage of Bacterial Cell Membranes by Pulsed Electric Field Treatment.”
2.30 pm	Break
2.45 pm	Christoph Schultheiss , Forschungszentrum Karlsruhe GmbH, Germany, “Industrial Applications of Electroporation”
3.15 pm	Hideyori Akiyama , Kumamoto University, Japan, “Environmental Applications of Biological Inactivation by Pulsed Electric Field and/or Pulsed Discharges in Water”
3.45 pm	Break
4.15 pm	Panel Discussion
5.30 pm	Adjourn

Abstracts of Invited Talks

Environmental Applications of Biological Inactivation by Pulsed Electric Field and/or Pulsed Discharges in Water

Hidenori Akiyama and Sunao Katsuki
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The phosphorus and nitrogen compounds flow into lakes and marshes near urban areas. In these nourishing surroundings, algae plankton increases and covers the water surface. Lakes often become the sources of tap water, and a kind of algae is poisonous. The protection of the environment at lakes and marshes is one of important subjects. Also, the sewage without enough treatment flows into a river and the seaside, and the destruction of water environments is caused. The sewage treatment is also one of important subjects. The protection of water environment is our obligation for descendants. The biological inactivation using pulsed electric field and/or pulsed streamer discharges in water is a potential method to clean the water. Here, some challenging trials to protect the water environment with pulsed electric field and/or pulsed streamer discharges in water are described and discussed.

Intracellular Electromanipulation of Mammalian Cells with Submicrosecond Pulsed Electric Fields

E. Stephen Buescher, Pamela S. Hair, Stephen J. Beebe, and Karl H. Schoenbach*
Eastern Virginia Medical School and *Old Dominion University, Norfolk, VA

The effects of high power submicrosecond pulsed electric fields on living cells have been examined. Theoretically, reducing the duration of high electric field (MV/m) pulses from microseconds to nanoseconds should increasingly affect intracellular rather than surface membranes of living cells. Experimentally, square-wave, 60 ns duration, 3.6 - 5.3 MV/m electric field pulses applied in trains of 1-10 pulses result in progressive increases in the numbers of permeabilized intracellular granules in a human eosinophil cell model - without large surface membrane effects. Electron micrographic examination of cells treated in this way demonstrates alteration of intracellular granule morphology consistent with permeabilization of granule membrane, i.e., intracellular electromanipulation. Continuous microscopic examination of individual living cells exposed to long or short duration pulsed electric field applications shows that rapid permeabilization of surface membrane (median 5 minutes) with anodic preference (electroporation) and prompt cellular swelling follow a single, long duration (100 ms) pulse. In contrast, after a single short duration (60 ns) pulse, onset of surface membrane permeability is delayed (median 17 minutes), the increased permeability shows no anodic preference, and cellular swelling is absent suggesting that these effects are due to intracellular electromanipulation rather than direct effects on the surface membrane. Submicrosecond, high power pulsed electric fields applied to living cells achieve preferential effects on intracellular rather than surface membranes, potentially providing new approaches for selective/generalized cell or tissue ablation, growth stimulation and tissue remodeling.

Dielectric Properties Of Biological Systems; From Amino Acids To Cells

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An interesting and important subject in biophysics is the investigation of the dielectric properties of complex biological systems like cells and their structural components (amino acids, proteins, membranes, cytoplasm etc.). These can bring valuable knowledge about the structure and dynamic behavior of proteins in solutions and different cell structures and their functions and metabolic mechanism in suspensions.

The aim of this contribution is to present a comprehensive theoretical and experimental study by means of time domain dielectric spectroscopy (TDDS) of static and dynamic dielectric properties of different biological systems including amino acids, regular and membrane proteins, normal and malignant blood cells of different types.

The dipole correlation functions for different globular proteins, such as myoglobin, lysozyme and RNase A solutions at different concentration, pH and temperature were obtained. The processes of the intramolecular interactions of myoglobin molecules, the pH-induced dimerization of lysozyme and thermal denaturation of RNase A are discussed. The dynamic structure study of native glucose oxidase and glucose oxidase, which was hydrophobized by covalent attachment of palmitoyl chains, is presented. We report also the application of (TDDS) to bacteriorhodopsin (bR) containing purple membrane films. The results of these measurements unexpectedly show that oriented purple membrane has unique liquid crystal like ferroelectric behavior. The dielectric behavior can be considered as a soft mode relaxation processes in ferro-electric liquid crystals near smectic-C* - smectic-A phase transition.

Suspensions of normal lymphocytes of T and B types and leukaemia lymphocytes (Raji, Daudi, Bjab, Peer and HDMar) were investigated at the low volume fractions (3-10%) where the cellular interactions are negligible small. The measurements were performed at a wide frequency range from 200 KHz to 3 GHz at room temperature 25 °C and physiological temperature 37 °C. The effect of electrode polarization in TDDS experiment was corrected. Cell radii and volume fraction were measured by independent methods.

It was shown that dielectric permittivity; capacitance and conductivity values of cell membrane are higher for normal lymphocytes in comparison with malignant ones. The difference of the same parameters between normal-B cells and normal-T is also discussed.

Dynamical Modeling of Cellular Response to Short High-Intensity Electric Fields

R. P. Joshi, Q. Hu and K. H. Schoenbach

Department of Electrical and Computer Engineering

Old Dominion University, Norfolk, VA 23529

The application of short, high-intensity electric pulses provides a mechanism for the non-thermal manipulation of biological cells. Some of the direct cellular effects include electroporation-induced variations in conductivity, ionic transport, shape deformation, localized energy absorption, and the onset of electro-chemical processes. It is already well known that the overall cellular outcome can either be reversible or irreversible depending on the characteristics of the external electrical excitation. An important first step for achieving a desired cellular response with as much control as possible, is to develop a quantitative understanding of electromagnetic field-cell interactions for such pulsed exposures. Previous simulation work pertaining to electric field effects on biological systems has generally been based on quasi-static models or invoked Laplace equation for the field distribution. In this presentation the dynamical aspects will be discussed. A comprehensive model at the cellular level that includes electrodynamics, changes in cell geometry, and electroporative variations associated with factors such as surface tension and pore area will be emphasized. A dynamical model allows for a better physical representation, and better agreement with observed experimental trends emerge. Implications for nanosecond, high voltage pulses will also be discussed.

Trauma and Injury Resulting From Strong Electric Field Exposure

Raphael C. Lee, MD, ScD, PhD (Hon)
University of Chicago, USA

Human use of electrical power is increasing. Along with that are increased opportunities for electrical injury. Victims of contact with high-energy electrical systems manifest a wide spectrum of injury depending on the electrical power capacity, current path through the body, duration of contact and healing response. Primary mechanisms of damage include membrane electroporation, electroconformational denaturation of membrane proteins and Joule heating. If arcing occurs, then acoustic blast injury can result. Skeletal muscle and nerves, tissues designed to be very sensitive to electrical signals, are particularly vulnerable to injury by the supraphysiological electric forces that are often associated with human contact with high voltage industrial power sources. Neurological and muscular damage often manifest despite lack of evidence of thermal burn. Severely injured victims present enormous medical management challenges due to release of inflammatory cytokines and altered cardiovascular behavior.

This paper will discuss the field exposure-tissue injury relationship and clinical manifestations of electrical injury. Approaches to rapid diagnostic imaging and therapy will be reviewed. In addition, the mechanism of action of promising new therapeutic amphipolar copolymers, which mimic behavior of natural chaperones, that have been shown to reduce tissue damage will be mentioned.

References:

1. Chen, W. and Lee, R.C. "Altered Ion Channel Conductance and Ionic Selectivity Induced by Large Imposed Membrane Potential Pulse" *Biophys. J.* **67**(2): 603-612, 1994
2. Mankani, M., Abramov, G., Boddie, A., and Lee, R.C. "Detection of Peripheral Nerve Injury in Electrical Shock Patients", *Annals New York Academy of Science*, Vol. 720, pp. 206-212, 1994
3. Marks, J., Pan, C., Bushell, T., Cromie, WJ and Lee RC, "Amphiphilic, tri-block Copolymers Provide Potent, Membrane-targeted Neuroprotection" *FASEB J.* **15**(6): 1107-9, 2001
4. Maskarinec, S.A., Hannig, J., Lee, R.C., and Lee, K-Y. "Direct Observation of Poloxamer 188 Insertion Into Lipid Monolayers" *Biophys. J.* **2002** *82*(3):1453-9
5. Occupational Electrical Injury and Safety. Chen, C.-T., Lee, R.C., Shih, J.-X., and Zhong, M.-H. Eds., *Annals of the New York Acad. of Science*, Vol. 888, 1999

Medical Applications of Electropermeabilization of Cell Membranes

Dietmar P. Rabussay

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Electropermeabilization (EP, also referred to as "electroporation") of outer cell membranes can be achieved by exposing cells to electrical pulses of field strengths between 100 and 2000 V/cm and pulse durations of 10^{-6} to 10^{-1} sec. EP is applicable to cells in suspension ("in vitro") as well as to cells in live tissue ("in vivo"). The optimal conditions for EP depend on many factors, including the properties of the target cells and their environment, the pulse parameters, and the geometry of the electrodes and their contact with the cells to be electroporated. EP has been used for about 20 years to deliver DNA and other molecules into cells *in vitro*, but its potential for medical applications was not realized until about a decade ago. Since then, a variety of medical applications have been explored in pre-clinical and clinical studies. The most advanced application, now in a Phase III clinical trial, is the treatment of malignant tumors. In this application, EP enhances the intracellular uptake of anti-tumor agents by tumor cells up to several thousand-fold compared to the uptake observed in conventional chemotherapy, and causes significantly improved tumor shrinkage or elimination. This cancer treatment modality has shown excellent potential for many different types of solid tumors. Another application of EP, the effective delivery of DNA into cells of several target tissues opens up the possibility of breakthroughs in gene therapy and DNA vaccination. EP appears to combine high efficiency, safety and economical feasibility, a combination that other competing DNA delivery methods have not been able to achieve. Experiments in large animals have confirmed the potential for clinical applications of EP in gene therapy and DNA vaccinations and the first clinical study is now in progress. Other *in vivo* medical applications of EP include many forms of vascular therapies (both drug and gene-based), treatment of glaucoma, and the use of EP-facilitated intradermal or transdermal delivery of drugs and vaccines to treat local and systemic diseases. Finally, EP has been explored as a technique to enable or enhance *ex vivo* therapeutic approaches. In *ex vivo* therapies, cells or organs are treated outside the patient's body before they are infused or implanted into the patient. Specific applications include the modification of blood clotting behavior, cellular immune therapies of cancer and other diseases, and prevention of organ rejection after transplant.

In summary, EP has opened up new approaches to solving numerous medical problems whose solutions were hampered by the difficulty of introducing drugs and genetic material across the barrier of the cell membrane. Some applications have progressed to advanced clinical trials and seem to fulfill the promise of this technology. Many other potential applications are still in early stages of development or have not yet been explored at all.

Industrial Applications of Electroporation

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Electroporation (EP) of biological cells has become a rapidly developing area of biotechnology. If external electric fields are applied across biological cells in a water suspension the cytoplasm becomes polarized. The charge displacement in the cytoplasm and in the suspension leads to high transmembrane potentials in the order of several volts. As a result large irreversible openings can built-up in the cell membrane, the cytoplasm can flow-out of and the cell can perish.

The subject of this talk is firstly to report on actual experiments at the mobile industrial electroporation pilot system KEA (Karlsruhe Elektroporation Anlage) with whole plants[1]. The maximum throughput of this device is 2 tons/hour. The reactor is a 16 cm-tube with integrated electrode pairs, where pulses with amplitudes up to 300 kV are applied at a repetition rate of 7 Hz. An overview of results from treated plants like beets, apples, grapes etc. will be given.

Secondly, design studies, development and test of an industrial EP production device will be presented. Since plants, as for example beets, are large in diameter and nearly weightless in water (suspension), the size of an electroporation reactor gap has to be large and the plants must be transported continuously by means of a forced feed-through. In the test facility ZAR (Zell Aufschluss Reaktor) a throughput in the order of 1 ton/min has been demonstrated. The operation of such a powerful device with a reactor spacing of 30 cm needs an assembly of Marx generators with high pulse repetition rates to establish fields at the reactor site as well as to fulfill power demands.

At Forschungszentrum Karlsruhe different types of Marx generators with gas spark gaps have been developed and tested. They are able to run with pulse frequencies in the order of 20-30 Hz (test Marx generator with 100 kV pulses). The pulse amplitude needed is 350 kV, the pulse length is 1 μ s and the pulse energy fed into a 20 Ohm load is about 1 kJ. The life time of the electric components is designed to withstand a minimum of up to 200 million discharge cycles (3 month operation). The complete system consisting of electroporation reactor plus conveyance is designed to be mainly built from dielectric materials to avoid undesired shielding effects of the electric field in the area of the reactor chamber. However high voltage strength dielectric materials like polyethylene or polypropylene underlie enhanced mechanical wear which must be taken into account.

1. C. Schultheiss, H.-J. Bluhm, H.-G. Mayer and M. Kern, "Industrial-Scale Electroporation of Plant Material Using High Repetition Rate Marx Generators", IEEE Pulsed Power Plasma Science 2001, Las Vegas June 17-22, 2001

Electrical and dielectric properties of experimental lipid bilayers in relation to biotechnology

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Transmembrane voltage pulses between 200 mV to 1 V are known to create transient 'pores' in the lipid bilayer, with a diameter about 2 nm. This phenomenon, termed electroporation, has been extensively investigated [1]. Electroporation occurs following electric pulses up to 10^6 V/cm with duration between μ sec and msec to membranes in close contact and is believed to initiate primarily in the lipid bilayer. This talk will begin with a brief summary of the origin of lipid bilayer research. One of practical applications of electroporation is cell transfection for gene expression. Other applications include encapsulation of drugs in controlled-release and insertion of proteins in living cells. For instance, the effects of the channel-forming peptide gramicidin D (gD) on the conductance and electroporation thresholds of BLMs have been reported [2]. It seems likely that the presence of membrane proteins affect the electroporation of the lipid bilayer by changing its mechanical properties. Transport of ions such as Na^+ , K^+ , Cl^- through membrane pores discharge the membrane potential, and at times an external pulse of sufficient amplitude and duration tends to cause dielectric breakdown of the lipid bilayer. Molecular transport through primary pores and pores enlarged by secondary processes provides the basis for transporting molecules into and out of cells. Tissue electroporation, by longer, large pulses, is involved in electrocution injury. Tissue electroporation by shorter, smaller pulses is under investigation for biomedical engineering applications of medical therapy aimed at cancer treatment, gene therapy, and transdermal drug delivery [3].

References

1. H. T Tien and A.L. Ottova, *Membrane Biophysics: As viewed from experimental bilayer lipid membranes (planar lipid bilayers and spherical liposomes)*, Elsevier, Amsterdam, 2000, 648 pp.
2. H.T. Tien and A.L. Ottova, The Lipid Bilayer Concept and Its Experimental Realization: From Soap Bubbles, Kitchen Sink, to Bilayer Lipid Membranes *J. Memb. Sci.*, 189 (2001) 83
3. A. Ottova, V. Tvarozek and H.T. Tien, Supported Planar Lipid Bilayers (s-BLMs) *in Planar lipid bilayers (BLMs) and their applications*, Elsevier, 2002, to be published

Electroporation of Biological Membranes from Multicellular to Nano Scales

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Several decades ago both cell and artificial planar bilayer membranes were found to exhibit dramatic changes if the transmembrane voltage was raised to 0.2 to 1 V by short pulses. Ionic and molecular transport were found to be increased by orders of magnitude, with both reversible and irreversible outcomes possible. Initially the term "breakdown" was applied. However, mechanistic interpretations presently exclude ion pair generation of classic dielectric breakdown. Instead, a transient aqueous pore hypothesis can account for many features of electroporation in bilayer membranes comprised of low dielectric constant constituents. My presentation will consider transient aqueous pore-based phenomena in planar bilayers, single cell systems often encountered *in vitro*, multicellular systems relevant to *in vivo* applications, and the expectation for significantly modified behavior for systems with very small spatial extent.

Damage of Bacterial Cell Membranes by Pulsed Electric Field Treatment

Ahmed E. Yousef¹, C. Patrick Dunne², Ragip Unal³ and Beatrice H. Lado¹
¹ Ohio State University, USA; ² US Army Natick Soldier Center, Natick, Massachusetts, USA; ³ Department of Food Engineering, Mersin University, Turkey

High-intensity electric pulses damage membranes of the microbial cell, leading to its injury and death. Based on this concept, pulsed electric field (PEF) emerged as a preservation technology to inactivate disease-causing microorganisms in food (Lado and Yousef, 2002). PEF treatment effectively inactivates bacteria and yeast in orange juice, milk, skim milk, yogurt, liquid egg and pea soup (Vega_Mercado et al., 1997); however, PEF is ineffective against bacterial and mold spores. This emerging food processing technology is developed with the promise to produce safe food and preserve its sensory qualities.

Lethality of PEF probably results from the electroporation of cell membranes. Formation of pores alters membranes' barrier properties, leading to loss of cell homeostasis. Membrane damage may be measured using fluorescence probes that gain entry only in cells with dysfunctional membranes. A fluorescent nucleotide-binding probe, Propidium Iodide (PI), was used recently to measure cell membrane damage during PEF treatment (Unal et al., 2002). According to this study, membrane porosity correlates well with cells' inactivation by PEF treatment. Therefore, measurement of membrane damage resulting from pore formation may serve as an index of PEF effectiveness against foodborne pathogens. Fluorescence staining technique showed that pre-treatment of gram-negative bacterial cells with lysozyme and EDTA increased the PI uptake by the PEF-treated cells. Therefore, it is possible to use the fluorescence staining technique to measure PEF-caused cell injury that is not detectable by cultural techniques.

References.

Lado, B. H. and Yousef, A.E. 2002. Alternative food preservation technologies: Efficacy and mechanisms. *Microbes and Infection* 4: 433_440

Vega_Mercado, H., O. Martin_Belloso, B.L. Qin, F.J. Chang, M.M. Gongora_Nieto, G.V. Barbosa_Canova, and B.G. Swanson. 1997. Non_thermal food preservation: Pulsed electric fields. *Trends Food Sci.* 8:151_157.

Unal, R., Yousef, A.E., and Dunne, C.P. 2002. Spectrofluorometric assessment of bacterial cell membrane damage by pulsed electric field. *Innovative Food Science & Emerging Technologies* (In press).

Poster Papers related to Dielectrics can be found in the CEIDP, 2002 Annual Report Conference on Electrical Insulation and Dielectric Phenomena, 02CH37372.

M. Stacey, J. Stickley, P. Fox, C. O'Donnell, K. Schoenbach, S. Beebe, and S. Buescher. "Increased cell killing and DNA damage in cells exposed to ultra-short pulsed electric fields" pp. 79-82

T. J. Lewis. "Electromechanical effects in biological membranes" pp. 87-90

N. Hassan, I. Chatterjee, N. G. Publicover, and G. L. Craviso "A combined experimental and computational analysis of membrane potential variations in excitable cells in response to DC electric fields" pp. 91-94

2002 IEEE/DEIS conference

The workshop was part of the 2002 IEEE/DEIS conference, which was held in Cancun, Mexico, October 20–24, 2002. The Conference on Electrical Insulation and Dielectric Phenomena (CEIDP) is sponsored by the IEEE Dielectrics and Electrical Insulation Society to provide an international forum for the discussion of work in progress on research on dielectric phenomena and measurements. The conference provides an opportunity for specialists from around the world to meet and exchange their findings.

Workshop Fee:

A conference fee of \$ 50 was charged to participants. This fee was used to cover expenses of documentation and beverages at coffee breaks.

Documentation

A book with the abstracts of the invited talks and copies of relevant publications has been provided to the participants. In addition, a special issue on “Biodielectrics” in the IEEE Transactions on Dielectrics and Electrical Insulation with J.C. Weaver and K.H. Schoenbach as Guest Editors will be published. The special issue will contain invited papers based on invited talks at the workshop and contributed papers.

Publicity

ELECTRICAL INSULATION

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January/February 2003



Workshop on "Dielectric Properties and Electrical Breakdown of Biological Membranes"

A one-day workshop on "Dielectric Properties and Electrical Breakdown of Biological Membranes" was held on 20 October during the 2002 CEIDP in Cancun, Mexico. The workshop was supported by the IEEE Dielectrics and Electrical Insulation Society, the US Air Force Office of Scientific Research, and the Office of Naval Research.

The membrane, which encloses biological cells, consists of a lipid bilayer with a thickness of approximately 5 nm. The dielectric constant is about 5, and its membrane resistivity is $2 \times 10^9 \Omega\text{cm}$. The function of the cell membrane is to isolate regions of different materials, but also to facilitate the flow of selected types of ions and molecules from one region to the other. Electrical breakdown of the membrane is defined as the onset of reversible or irreversible changes in the cell membrane (electroporation) and occurs at membrane voltages of greater than 1 V, which corresponds to electric fields of greater than $2 \times 10^6 \text{ V/cm}$. A group of leading scientists in the field of biodielectrics presented invited talks on membrane structures, membrane functions, dielectric properties, and electroporation. In addition, an overview of applications of both reversible and irreversible electroporation in medicine and biology was presented. This included gene therapy, cancer treatment, biofouling prevention, and bacterial decontamination.

The workshop was concluded with a panel discussion.

Obviously, there is a strong interest on the part of the DEIS community in this research area at the interface of engineering and biology. This was manifested by the very positive feedback of the approximately 50 workshop participants when asked about the content and format of the workshop, and by the decision of the biodielectrics subcommittee to offer a follow-up workshop in biodielectrics at the 2003 CEIDP in Albuquerque, NM. Another opportunity to learn about, and to participate in, biodielectrics is the publication of a special issue on the topic of the 2002 workshop in the *IEEE Transactions on Dielectrics and Electrical Insulation*. Guest editors are J. Weaver (MIT) and K.H. Schoenbach (ODU). The deadline for submission of manuscripts is 15 January 2003.

—This report was written by
Karl H. Schoenbach,
Chairperson of the Workshop.



Karl H. Schoenbach, summarizing the Workshop on Biological Membranes.

Announcing a Special Issue on

Dielectric Properties and Electrical Breakdown of Biological Membranes

in the
IEEE Transactions on Dielectrics and
Electrical Insulation

with
J.C. Weaver and K.H. Schoenbach
as Guest Editors.

Deadline for Submission: January 15, 2003
Information for authors can be found in any recent issue of the
IEEE Transactions on Dielectrics and Electrical Insulation.

Please mail manuscripts to
either

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